

British Society for Antimicrobial Chemotherapy

Standardized Disc Susceptibility Method

User Day Report

for

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Meeting

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1 Introduction

Mrs Andrews began the proceedings by welcoming the audience to the Queen Elizabeth Medical Centre. She continued by saying that the programme was slightly different to those in the past that had concentrated on technical issues. Today the first speaker, Professor Wise, would be giving lecture on antimicrobial resistance, followed by Dr Dryden discussing the clinical applications of susceptibility testing and that after lunch the afternoon session would be devoted to a round table discussion. She then introduced Professor Richard Wise the Chairman of the BSAC Working Party on Sensitivity Testing.

2 Antimicrobial Resistance - Professor Richard Wise

Antimicrobial resistance is in many ways what lies behind susceptibility testing. It is one of the major reasons to find out whether an organism is likely to respond to therapy or otherwise and also to collect information on microbial resistance. Antimicrobial resistance is a major problem to society and history shows that it is an increasing problem. There is a distinct lack of new agents coming along the antibiotic pipeline and over the last 25 years only one new class of agents has emerged, linezolid. More lives have been saved with antibiotics than any other group of therapeutic agents, but these drugs are now considerably endangered. This is because of antibacterial resistance.

Two years ago The House of Lords Select Committee on Antimicrobial Resistance produced a report and in the opening paragraph stated '*It was an alarming experience, resistance constitutes a major threat to the public health and ought to be more widely recognised than it is at present.*' Governments around the world have become aware of the fact that the problems are extraordinarily complex and very difficult to deal with, requiring a change in human nature, which is never very easy to do, on how to prescribe and how people make decisions. There has been another House of Lords Report and now there is a Specialist Advisory Committee on Antimicrobial Resistance to the DoH. In fact the Swann Committee recommended this years ago in 1969.

The bacterial world has probably 10^{28} bacteria in contact with mankind. That is the organisms in the body, on skin, in gut, multiplied by the population of the world, taking into account the animal reservoirs we are in contact with. This is a number that is difficult to comprehend.

Mankind have roughly the same mutation rate as bacteria say $10^{7/8/9}$ on the DNA, but bacteria have far more sophisticated ways of exchanging information, compared with man who reproduces once or twice every 25 years, with only one method of reproduction and dissemination of genetic information.

Antibiotic use worldwide is extremely hard to ascertain, but is reckoned that it is about 100 million kgs +/- twofold. This may be grossly inaccurate because in China alone there may be 50 million kgs produced. Selection pressure is incredible and is not surprising that resistance emerges. Organisms carrying a resistance gene have the advantage that when exposed to antibiotic they survive and will fill that ecological niche whether that is in the human being or the environment, and will grow out spreading its information vertically. A good example is pre-Fleming when *Staphylococcus aureus* was fully susceptible, but with the advent of the penicillins in 1950s the organism very rapidly became penicillin resistant. Beechams introduced methicillin and in the '70's and 80's and MRSA appeared, and is still causing problems today. Vancomycin has been available for a long time, but its usage is increasing considerably and now in year 2000, Hiramatsu in Japan described the first vancomycin resistant *S. aureus*. As night follows day resistance follows use and abuse.

Bacteria can become resistant to antibiotics by producing antibiotic altering enzymes such as aminoglycoside modifying enzymes; antibiotic degrading enzymes for example β -lactamases; efflux pumps such as those found in quinolone resistant organisms; or alterations to cell walls preventing the penetration of the antibiotic. There are a number of mechanisms and each antibiotic has its own mechanism of resistance associated with it. There is a plethora of mechanisms for resistance and also for exchanging genetic information. In the plasmid method a resistance gene from one organism can be transferred to an organism of the same bacterial species or a different genera. Connected with these are transposons on the plasmids, or integrons in gene cassettes on the transposons; this is a very sophisticated way that

bacteria can rapidly exchange genetic information. Bacteria can take information from a dying lysing cell, called soluble (something of a misnomer) DNA and this material is integrated into the chromosome. This is the mechanism of resistance of *Streptococcus pneumoniae* to penicillin; probably DNA from oral streptococci has been transferred into the pneumococcus and integrated into the chromosome causing a resistance problem. Bacteria can also gain genetic information via viral delivery by bacteriophage. As the bacteriophage chromosome goes into the bacteria, if it contains bacterial resistance information, it can integrate with the bacterial DNA and possibly cause resistance. This occurs in staphylococci, pneumococci, and Enterobacteriaceae (particularly adept at this mechanism of resistance).

Resistance is an increasing, accelerating and accumulating problem. Virtually all classes, whether they are bacteria, viruses, round worms, trichomonas or fungi show this problem. Resistance, once it occurs, is extraordinarily difficult to tackle and reduce.

Antibiotics are used roughly equally between human and animal medicine. In the case of humans around 95% is used in general practice and only 5% in hospitals. Antibiotics are used a great deal in general practice and 60% to 70% of the use is questionable. In the case of animals, 80% is really used for commercial purposes, as growth promoters or it has been commented, to overcome the problems of poor animal husbandry. Altogether 70-80% of all antibiotic use is highly questionable and should or could be reduced. There are even more dreadful uses of antibiotics than in animals:

1. Five hundred grams of tetracycline treats 500 patients, but in the USA 50,000 lbs of tetracycline is used to spray fruit trees to prevent a disease called 'Fire Blight', caused by an erwinia organism. Streptomycin is also used and it has been observed that isolates of erwinia are now becoming resistant.
2. In the USA and Norway 150 lbs per acre of antibiotics are put into salmon farming lakes. Often these are fluoroquinolones, antibiotics that are particularly useful in man. Scotland are trying to reduce the amount of fluoroquinolones used.
3. In Canada there is a disease of lobsters called Gafkyinia, an organism like the staphylococcus, and very large amounts of vancomycin are used to treat this disease.
4. Disinfectants are one of society's answers to the big problems of resistance. Chopping boards are impregnated with disinfectants; most household cleaners have antibacterials in them. This is not the answer to any of the problems because there is a linked resistance to certain agents, for example triclosan and other agents used in these disinfectants. So by heavy use of disinfectants there is increased selection pressure for antibiotic agents as well.

Hospital intensive care wards are the most active place in the hospital environment, where there is heavy antibiotic use, opportunities for cross infection, natural selection in action and antibacterial resistance occurring. The community is of greater concern because it is worldwide.

Methicillin resistance in *S. aureus* is a worldwide problem with 30-40% of isolates resistant to methicillin (varies between hospitals). It is causing major financial and political issues worldwide. MRSA was initially a hospital pathogen but is now spreading into the community. A study done in San Francisco showing about a quarter of nasal swabs were positive for *S. aureus* and 12% of those were MRSA and giving some risk factors here. A recent publication from Finland again showed 21% of their staphylococci were MRSA and community acquired.

Other problems that may arise in the next year or so and some that have been observed already are as follows:

I Vancomycin resistant staphylococci (VISA)

Glycopeptide resistance was first observed in Japan and there have been further isolates around the world; a few in the USA; Southmead Hospital in Bristol described a heterogeneous resistant isolate; Central Europe; Addenbrookes Hospital in Cambridge have recently isolated a homogeneous resistant organism. No evidence of spread, but of concern if resistance in these organisms increases.

2 Vancomycin resistance in enterococci

Increased incidence, and equally common, in hospital intensive care and non-intensive care units. In the UK the rate is higher in the specialist units such as liver transplant units.

3 Carbapenemases

Carbapenemases have been described in the Far East. Usage of carbapenems in the UK is moderate. However, increased use of imipenem, meropenem and ertapenem (to be marketed later this year), will increase the selection of carbapenemases.

4 Extended spectrum beta lactamases (ESBLs)

New types identified in the Far East now being found in the UK. Need to be sure that we can recognise these organisms.

5 Fluoroquinolone resistance in S. pneumoniae**6 Increased penicillin resistant S. pneumoniae****7 Fluoroquinolone resistant Haemophilus influenzae**

Resistance to the fluoroquinolones is rare, but appearing, and will become an increasing problem as the fluoroquinolones are used more in children.

8 Antiparasitic

Resistance to metronidazole has been described in Trichomonas vaginalis. Cattle round worms resistant to the drug mabenzole and similar agents have been described and are going to become a major veterinary problem.

9 Antiviral resistance

In HIV in South Africa.

10 Antifungals

Problems currently only seen in HIV patients.

2.1 Way of tackling these problem areas:**2.1.1 Reduction in use of antibiotics**

From a study in New York where there was a high incidence of cephalosporin resistance, the use of these antibiotics was restricted. A reduction in use of 80% compared with the previous year was accompanied by a 44% reduction in the incidence of ceftazidime resistant Klebsiellae. However, as imipenem was used to treat patients, a 69% increase in imipenem resistance was observed. This illustrates that controlling usage in one area creates a problem in another.

2.1.2 Use of second line antibiotics

The use of the anti-staphylococcal agent fusidic acid has doubled in the last five years with a simultaneous tripling of resistance. It has been used, abused and overused in the dermatological setting.

Some resistance issues can spread from animals to man, not a major issue but it can happen. In human and animal setting there is cross infection. The solution is easy in theory, improve infection control, reduce antibiotic pressure by reducing antibiotic prescribing and cut out unnecessary use in animals. This is far easier said than done. There is a need to education parent, doctors, teachers, vets and farmers. Education and legislation are needed to remove certain food additives and growth promoters. Communication, research, the production of new agents, underpinned by surveillance of resistance rates by the laboratories is needed. It is irrelevant which standardized method of susceptibility testing is used (Stokes's comparative method of testing is not a standardized method and is therefore not recommended for surveillance of resistance studies) as long as the method is robust. This is essential for surveillance of resistance studies.

2.2 Use of antibiotics in the community

Globally, 400 million prescriptions in 1999 out of a total 800 million were for antibiotics. Twenty percent of these prescriptions were for 'acute unspecified upper respiratory tract infection', presumably a cold.

- Tonsillitis, pharyngitis, trachyitis - a cold going down onto the chest.

- Acute bronchitis- usually a viral disease that does not need to be treated with antibiotics.
- Sinusitis- unless present for 8 days or associated with acute pain, tenderness over the sinuses, does not require antibiotics.
- Otitis media- does not require antibiotics.
- Community acquired pneumonia- **does** require antibiotics.

The vast majority of these conditions do not require antibiotic therapy, so campaigns are often used to educate doctors and patients. Campaigns for public health have to be hard hitting and repeated year on year to have any effect, and last winter The Department of Health organised a campaign called 'Antibiotics don't work' that targeted mothers and children. The success of this campaign has encouraged the targeting of another group; children and teachers in secondary schools, for whom packs are being produced (today's children will be the parents of tomorrow).

2.2.1 Results of the audit of the last campaign:

- Fifty percent of people have had an antibiotic in the last 2 years.
- There were more in social groups C2 downwards than in A, B or C1.
- Sixty percent of the antibiotics prescribed were for upper respiratory tract infections (the UK is the same as the rest of the world).

An American idea that has had a moderate degree of success is the use of non-prescription pads. Patients like to come from a GP with a piece of paper, so this non-prescription states '*no antibiotic needed*' and gives some advice including that if the condition worsens then the patient should return to the surgery.

2.2.2 Some of the questions asked of people:

Bacteria are becoming resistant:

a large majority people said they had heard about this.

Antibiotics weaken the body's resistance to infection:

untrue but many people believe this to be the case.

Antibiotics are prescribed too readily:

people seem to think that, but expect to have them prescribed by their GP.

Antibiotics cure most things:

most people seem to think they do.

Do patients trust their GP when they are prescribed antibiotics:

most people trust their GPs, but there is a miss match because GPs tend to think that patients want antibiotics but some of these data does not support this.

What do you think an antibiotic is prescribed for:

20% expect it for a sore throat, for a bad cough etc.

2.2.3 How much can antibiotic use be reduced in man?

Data presented has shown that ideally, 75% or more could be reduced. It would be very difficult to reduce the amount of antibiotic usage because of patient and doctor expectations and the pressures of the pharmaceutical industry upon doctors to prescribe. Soon the EU may be allowing advertisements by pharmaceutical companies direct to potential patients, alerting them about the use of different drugs. The numbers of antibiotic prescriptions in the UK are coming down. There has been a 21% fall since the peak in 1995, but there is also a six-fold difference between one practice and another indicating that there is a lot of room for improvement. General practitioners seem to think that resistance does not affect their practice and they have this perception because they are not looking for it. Doctors have got to change their attitudes to how antibiotics are used. Traditionally, the oldest and cheapest drug was used first. Another approach could be to 'use shorter courses, higher doses, possibly of a more active compound rather than the least active. There are three diseases where combination therapy is mandatory because of resistance issues TB,

HIV and malaria. These are major worldwide problems. Should combination therapy be used more often perhaps in the case of other killing diseases such as community-acquired pneumonia?

The cost of antibiotic development is increasing tremendously and if resistance develops rapidly then it is a poor return on the investment. This is the preliminary thinking to not starting at all.

We have to do something, other wise we will be back to putting maggots on wounds.

2.3 Questions

- Q** **Dr Dryden:** I am sure we all believe the philosophy that we need to reduce antibiotics and need to reduce the selective pressure on bacteria, but on the other side since the recent public health programme has taken place, I am sure I have seen more serious infections, say with *S. pyogenes* in children and acute mastoiditis for example, which I have not seen for about a decade. I think the GPs in our area have been reducing antibiotic usage for common viral infection, but I suspect there may be a small price to pay. Individuals may get a more serious infection because they have not been given an antibiotic.
- R** **Professor Wise:** This is a very difficult tight rope to walk, reducing antibiotics and not putting people at risk. I think one can only do this by trying to dissect out which patients are more at risk than others. I have not seen such data published, however, there was a publication about community-acquired pneumonia in the British Thoracic Society's Journal, stating that patients were more severely ill if they had not received antibiotics from the GP.

3 Clinical applications of susceptibility testing - Dr Matthew Dryden

Dr Dryden gave a broad presentation on the rationale for susceptibility testing including the BSAC method and the clinical applications of these tests. He began by using the events that had occurred to a 30-year-old SAS sergeant, admitted on 6 January to Winchester Hospital with a fever of unknown origin, he showed how susceptibility testing results helped to explain initial clinical failure, and the subsequent management of the patient.

3.1 Background

- 3.1.1 Illness began in Oman, however he had spent time in Afghanistan before the main wave of infiltration.
- 3.1.2 Debilitating swings of fever and sweating for about three weeks (tough individual who had continued to work).
- 3.1.3 Diarrhoea initially, followed by constipation.
- 3.1.4 Shortness of breath on exercise, but not at rest
- 3.1.5 Diagnosed with influenza and prescribed Relenza.
- 3.1.6 After one week of taking Relenza he continued to suffer sweats and a general feeling of tiredness.
- 3.1.7 Relenza stopped. Prescribed a course of ciprofloxacin to which he did not respond.

3.2 On admission on the 6 January

- 3.2.1 Went to the local A&E department at Winchester when on leave in the UK.
- 3.2.2 On examination nothing abnormal found, but admitted for further tests.
- 3.2.3 On monitoring his temperature a high swinging fever was found.
- 3.2.4 Chest X-ray normal.
- 3.2.5 In the previous year had travelled to Sierra Leone, Malawi and the jungle in Brunei. The differential diagnosis could have been anything (had probably been exposed to mosquitoes, ticks and there are leeches in Brunei), however malaria was suspected and a registrar prescribed quinine (ideally, treatment should have withheld until investigations were complete).

3.3 Blood tests

- 3.3.1. Platelet and white count normal; haemoglobin slightly low; slightly raised CRP, creatinine and alkaline phosphatase; numerous blood films for malaria (all negative); serology for Leptospirosis, Schistosomiasis and Brucella, respiratory viral screen and hepatitis (all negative); malaria serology positive(although he had been on malaria prophylaxis on and off throughout the year).

3.4 Microbiology

- 3.4.1 Stool samples requested, but difficult to obtain because of constipation.
- 3.4.2 Three sets of blood cultures taken. Gram stain of the third blood culture revealed a pleomorphic Gram-negative cocco-bacillus with morphologically similarities to haemophilus. Following day a ciprofloxacin resistant non-lactose fermenter that agglutinated *Salmonella paratyphi* antisera, later identified as *Salmonella paratyphi* A, isolated. Ciprofloxacin resistant by BSAC disc methodology. MIC of 16 mg/L by Etest.

3.5 Diagnosis, management and outcome

- 3.5.1 Enteric fever. Paratyphoid A.
- 3.5.2 Malaria antibodies positive. Quinine treatment was continued (having been started empirically) followed by Fansidar.
- 3.5.3 IV ceftriaxone (to which the organism was very susceptible) once daily as an outpatient.
- 3.5.4 CRP came down and temperature settled.

That was an example of the BSAC disc testing method showing a reduction in the susceptibility of the infecting organism to ciprofloxacin. It fitted well with the fact that the patient had not responded to ciprofloxacin treatment in the Middle East and ultimately responded to an antibiotic to which the organism was very susceptible. Isolates of *S. typhi* and *paratyphi* resistant to ciprofloxacin are well described in the Middle East. It is possible that sub therapeutic doses have been administered and organisms have become resistant to ciprofloxacin.

3.6 Use of susceptibility testing in a diagnostic laboratory

Diagnostic laboratories use susceptibility testing as an aid to predicting clinical outcome in individual patients. However, susceptibility testing is also undertaken for surveillance of resistance patterns, to build data bases of information on particular diseases, to predict the best antibiotic for empirical treatment and to help in setting antibiotic policies. Antibiograms were also useful for identifying certain organisms, such as *Stenotrophomonas*, and also as an epidemiological tool in outbreaks of cross infection.

3.7 What method should be used for susceptibility testing?

With regard to susceptibility testing there was a philosophical divide. Should the same method be used worldwide or should an effort be made to use the same method in a country or continent. There are clear advantages in that because using the same method means that more value comparisons between sites can be made. Equally, scientific ingenuity should not be stifled so that new mechanisms of resistance are not missed. Whatever method is used for a diagnostic laboratory it must fit the following criteria:

3.7.1 Simple

3.7.2 Cost effective

3.7.3 Cope with the workload

3.7.4 Reproducible

Disc testing methods were often preferred because it enabled the examination of zones of inhibition, allowing the detection of resistant micro colonies or mixed infections when direct susceptibility tests were performed.

3.8 Examples of the disc testing method being used to detect mixed infections

3.8.1 Case 1

A young man in his 30s with a neuro-endocrine tumour and liver metastases who was septicaemic. The Gram stain of the blood culture showed Gram-negative rods and Gram-positive cocci in short chains and pairs. Direct sensitivity testing plates set up for testing Gram-negative organisms to meropenem, amikacin, gentamicin, piperacillin, ciprofloxacin and ceftazidime, differentiated an enterococcus and a Gram-negative organism that subsequently was identified as *Citrobacter diversus*. Detection of this mixed infection may not have been so easy to recognise if a microtitre or breakpoint method of susceptibility testing had been used.

3.8.2 Case 2

Patient with enterococcal endocarditis.

Disc testing to gentamicin revealed a sub-population growing up to the gentamicin disc. Treating this patient with combination therapy would be extremely difficult.

3.9 What evidence is there for susceptibility testing predicting the success of treatment?

Two clinical cases were presented to illustrate outcome related to susceptibility results. In the first case, a female with a urinary tract infection was treated empirically by her GP with trimethoprim. Subsequently a coliform resistant to amoxicillin and trimethoprim was isolated, yet 1 week later the symptoms had resolved and a urine culture was negative. In the second case, a fully susceptible *E. coli* and anaerobes were isolated from liver pus and blood cultures. Despite adequate antibiotic treatment (combination of ceftriaxone, gentamicin, metronidazole) the patient died.

So two opposing examples, the first the organism was reported resistant yet the patient gets better very quickly, second example the organism is fully sensitive, patient had lots of antibiotics and yet he died from overwhelming infection. So why did the susceptibility results fail to predict the outcome in these patients?

It is obvious when you think about it. There are many factors that influence whether a patient responds to antibiotics, and not just whether the organism is susceptible or not. There are a huge number of host factors, the severity of the disease, underlying immunodeficiency, the site of the infection, all these things are very important for the survival of the patient. In the pre-antibiotic era, individuals had bacterial infections and by and large most of them survived, but occasionally if the infection was severe they died. The description of pneumococcal pneumonia in the pre-antibiotic days was one of 'crisis or lysis'. The crisis was death from overwhelming infection and respiratory failure, lysis was gradual recovery as the organisms were destroyed by the immune system and the inflammatory material was reabsorbed from the lungs and lung function improved.

There are pharmacological and pharmacokinetic considerations that have to be taken into account:

- i. Maximum concentration of antibiotic at the site of infection.
- ii. Half-life of the antibiotic.
- iii. Area under the curve.
- iv. Time of the concentration above the MIC.
- v. Degree of protein binding
- vi. Whether the antibiotic is metabolised, and if the metabolites are active microbiologically.

All of these things are important in clinical outcome and makes defining breakpoints very difficult. However, the BSAC formula that was first described in the 'Guide to Sensitivity Testing' in 1991¹ has attempted to address all of these concepts when devising *in-vitro* breakpoint concentrations.

Perhaps definitions should be modified as follows:

Organism considered susceptible -

antibiotic will contribute maximally to the therapy taking into consideration all the other variables.

Organism considered resistant -

antibiotic should make no contribution to therapy

Organism considered to intermediate susceptibility -

a range of intermediate contributions from the antibiotic can be expected.

3.10 Scientific evidence to support the correlation between susceptibility and clinical outcome

The scientific evidence to support the correlation between susceptibility testing results and clinical outcome is very limited. In the cases of simple infections such as gonorrhoea, a bacterial infection of the mucous membranes, it is relatively easy to give an antibiotic and then detect if the infection is cleared and if the patient gets better. There is good scientific evidence to show that if *Neisseria gonorrhoeae* produces β -lactamase there is an extremely high failure rate (99%) if ampicillin is used to treat the infection, compared with a high cure rate if the organism does not produce β -lactamase (1%).

Another example of a simple infection is CAPD peritonitis. In this instance infection is in one area of the body and antibiotic is administered directly to the site, so the only variable factor is the susceptibility of the organism. Coagulase negative staphylococci are a common cause of CAPD peritonitis. From personal research it was observed that when ciprofloxacin was used for treatment, all of the treatment failures were in patients where organisms were resistant with ciprofloxacin MICs greater than 4 mg/L. In this study there was a very good correlation between the MIC and the success rate of treatment with ciprofloxacin. In simple infections like gonorrhoea and CAPD peritonitis the antibiotic plays a major dominant role in the outcome of infection. In more complicated infections with more variables, such as uncomplicated UTI and exacerbations of COPD, an antibiotic certainly contributes. For very complicated infections the antibiotic

plays a partial contributory role. In abscesses you have to get the pus out and do the supportive treatment as well as give the antibiotic.

Ibrahim² and Leibovici³ have shown that mortality in septicaemia is greater if the patient is given an antibiotic not active against the infecting organism (mortality rate for inadequate and adequate treatment 61-62% and 28.4% respectively) or if the wrong antibiotic is given (34%).

At Winchester hospital an audit has been undertaken to analysis the septicaemia data over a 10-year period (2,500 cases of recorded bacteraemia septicaemia). Where patients were given inappropriate empirical antibiotics the mortality rate was 19%. When appropriate antibiotics were given the rate was below 10%.

A study by James Burnie⁴ has shown that the mortality in MRSA septicaemia depends on empirical therapy. Mortality was low (4%) if the patient was given vancomycin plus rifampicin (1 death out of 25), but mortality was higher (38%) if the same combination was given and the organism became resistant to rifampicin during therapy. If the patient was given vancomycin alone or the organism was rifampicin resistant at the time of initiation of therapy, the mortality rate was incredibly high (78%). This study was aimed at the use of combination therapy with MRSA and also to show that susceptibility does matter when treatment is started.

3.11 The use of susceptibility testing in the terms of surveillance data and epidemiology

In 1995 Winchester undertook an audit looking at the types of organisms isolated from community versus hospital-acquired septicaemias. In general, the organisms isolated from community-acquired infections were more sensitive than those acquired in hospital, for example 40% of Gram-negative organisms isolated from the community were resistant to amoxycillin compared with 60% in the hospital isolates. There was no ciprofloxacin resistance in community-acquired infections whereas in hospital acquired infections resistance was emerging.

In Winchester, there is on-line prescribing. The antibiotic policy on the system is designed to allow junior doctors to get the most appropriate empirical therapy for patients when a clinical diagnosis is made. For example, in the case of a patient in A & E diagnosed with septicaemia the physician clicks onto septicaemia. A list of types of septicaemia ie with urinary focus, respiratory focus etc is then revealed. Clicking on the appropriate focus of infection gives information about the organism likely to be causing the infection and a choice of three antibiotics to prescribe empirically. It is a good guidance system although not always used.

Another audit for the medical department in 1994 looked at the choice of empirical antibiotics chosen for septicaemias and its effectiveness for the diagnosis. The following definitions were applied:

Correct - the organism was sensitive and the agent was the most appropriate for that diagnosis according to antibiotic policy.

Appropriate - organism sensitive and agent should be effective.

Inappropriate - antibiotic unlikely to work because the organism was resistant or not suitable for that diagnosis.

204 septicaemias over the course of a year in the medical unit were studied. The majority of patients (82%) got an effective antibiotic. Slightly over half were given the correct antibiotic according to the policy, 28% were given appropriate antibiotics that would have treated that infection, and 18% were inappropriate. In the appropriate group there was a tendency to over treat (defensive medicine). In order to give the patient the best chance possible, multiple antibiotics are given to treat all eventualities, but this should be modified to reduce the selective pressure exerted on hospital organisms. Lines are a common cause of infection and staphylococcus and coagulase negative staphylococci were some of the inappropriately treated septicaemias.

3.12 Other specific infections that are inappropriately treated

Pneumonias in hospital are possibly over treated. Patients come into hospital with a community-acquired pneumonia and receive a broad-spectrum cephalosporin plus a macrolide, both given IV and both expensive. The majority of cases of lobar pneumonia are caused by pneumococci and at Winchester there is virtually no pneumococcal penicillin resistance and therefore, penicillin is still the drug of choice for pneumococcal pneumonia in the hospital antibiotic policy. It is possible, but unlikely that there is a coexistent Chlamydia or Mycoplasma infection that justifies combined treatment with a macrolide. The majority of patients do get a cephalosporin; the newer ones do have good pharmacokinetics, and have good levels in epithelial linings and alveolar fluid, so it could be argued that they are a better choice of antibiotic. Nevertheless, penicillin does work well in most cases of pneumococcal pneumonia and a cheap and effective antibiotic should be used wherever possible, underpinned susceptibility testing to monitor levels of resistance.

3.13 What do you do in cases where there is no organism for susceptibility testing?

There are some instances where organisms cannot be grown and therefore susceptibility testing cannot be undertaken. The results of therapeutic success have been used to establish the antibiotic most appropriate for treatment. In the case of *Borrelia* (Lyme Disease) that is quite prevalent in Hampshire, diagnosis is based on serology results, but there is no information on whether the organism is becoming resistant to the standard treatment of either a β -lactam (amoxicillin or ceftriaxone) or tetracycline (doxycycline used in adults).

3.14 Correlation of the results of susceptibility testing by the BSAC method with clinical response

3.14.1 Case 1

Like many of you here today we use the BSAC method for the detection of methicillin/oxacillin resistance in staphylococci. Generally, we find either large zones of inhibition for the susceptible population or no zone of inhibition for those organisms with MICs of >256 mg/L. Recently we had an isolate from a Special Baby Care Unit (SCBU) with a reduced zone of inhibition by BSAC testing. By Etest the MIC was 16, so it was a genuine MRSA with different sensitivity pattern from our MRSA15s. This was the first of an outbreak of MRSA on SCBU that included a septicaemia in a baby, so this was highly relevant. The BSAC method picked this up and correlated well with the methicillin MIC.

3.14.2 Case 2

Most of the patients with urinary associated sepsis go onto cefuroxime. The direct Gram-negative sensitivity plate from the blood culture showed the organism with a zone diameter very close to the susceptible zone diameter breakpoint. The patient's temperature was still spiking and he was pretty unwell, so on the basis of susceptibility testing the antibiotic was changed rather than waiting another day.

3.15 Conclusion

Susceptibility testing is very useful in enabling microbiologists to forecast the outcome of therapy, it allows the development of data to look at the epidemiology and trends of bacterial infection; it allows us to develop empirical guidance for therapy and occasionally allows us to identify organisms quickly.

3.16 References

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3.15 Questions

- Q** Using a Vitek what sort of impact do you think rapid methods would have in treating, getting a result in 6 hrs rather than waiting a day.
- A** I am not sure it is going to make a huge difference. I like the idea of the very scientific molecular methods for predicting methicillin resistance or Tem genes. Again it is hard to tell how major a clinical impact is going to have.
- Q** I have used the Vitek at Alder Hey where I used to work and what you said about the agar plate and actually seeing the bacteria on the plate, you don't get that with the card system. I think it can be very useful when you can see the mixture; I have great reservations about the Vitek.
- R** **Matthew Dryden.** You have the purity plate with the Vitek and next day you know if it is pure.
- R** Yes, but with the plate sensitivity you get an idea of what is going on and can guess what is going on.
- R** **Matthew Dryden.** I presume if you have a mixed blood culture like one of the first ones I showed you, you have to get a purity plate of both organisms before you can inoculate your Vitek card.
- R** Yes so the result from putting it on the machine is quicker, but getting the specimen ready for the machine is slower and more difficult.
- Q** I know there has been some work done with cystic fibrosis patients looking at mixed cultures for sensitivity because you are possibly getting an idea of what is happening in vivo. Have you any thoughts on that?
- R** **Matthew Dryden.** We have quite a few cystic children we treat in Winchester and Southampton microbiology, down the road from us, have an interest in cystic fibrosis. Southampton has a bench that looks at mixed cultures and synergy testing on cystic isolates. Cystic fibrosis specimens can present with different sensitivities on different days it is very difficult to interpret the findings from these patients.

4 Afternoon session general discussion

4.1 Professor Wise

At the first meeting of the working party Professor Ian Phillips sent us all away with the task of thinking about how could we reach reasonable, meaningful breakpoints? I had a Garrod and O'Grady book with decent sets of tables in, and managed to work out a rough formula that took into account the C_{max} , protein binding, half-life and it seemed to work. Ian Phillips added the 'f' factor, where you move things around in order to avoid a breakpoint ending up on the middle of a distribution curve of MICs. There is a move to change the breakpoints again to fit in with pharmacokinetics and pharmacodynamics. The BSAC formula does in fact take that into account. Now the Americans are moving to choose MIC breakpoints closer to the BSAC (UK) recommendations than their original BPs. There are various reasons why NCCLS BP were higher. Firstly, industry has a major input into NCCLS, whereas at the BSAC we have only a nominal input. In industry they want BPs to be high, so that there are an enormous amount of bacteria in the remit, we tend to be more conservative.

The BSAC method is published and the website is available for reviewing 4 to 5 times a year and there are some changes to follow shortly. It is a changing process needed for continual improvement. There are gaps that we need help with.

How does one set a breakpoint to a drug such as cefuroxime where the breakpoint sits in the middle of the MIC distribution curve? What do you do about co-amoxyclav with two components? Do you have a ratio of the two drugs or a fixed concentration of clavulanate and just alter the ratio of amoxycillin? There are no definitive views on this and we sometimes change our opinions while we are trying to get matters right.

4.2 Discussion

- Q** Last time a sheet was given out related to testing fastidious organisms by BSAC methodology. We are interested in the Campylobacter method, that we followed incubating at 37^o, but did not have much success in getting the organism to grow well and we were not able to read the results on our Mastscan system. Can you help in any way?
- A** **Jenny Andrews.** You used Anna King's recommendations? I think you should be incubating at 42^o C. Perhaps you should contact her directly.
- Q** We have a problem with QC results for Mast clindamycin discs when checking the control Staphylococcus. The values are on the lower edge of the range you were giving.
- A** **Jenny Andrews.** What is the depth of your media are you sure it is 4 mm depth?
- R** We usually work with 90 mm plates with 25 mL of agar.
- R** **Jenny Andrews.** Have you tried looking at another manufacturers discs? It has been noted that there are differences between discs from different sources. Would it be possible for you to send me your results?
- R** **Professor Wise.** There are some problems with Mast discs that we know of but not to clindamycin. If you can give us comparison data on two sets of discs eg Mast and Oxoid it will be very valuable resource to us.
- C** **from the audience.** At the last user group meeting in London someone in the audience mentioned that they had difficulty with Mast discs because for ciprofloxacin they were consistently getting zones below them lower limits for the control strains. Mast has been looking at ciprofloxacin as you mentioned and presently they are analysing the content of their ciprofloxacin discs in comparison with discs from other sources. It has also been noted with other antibiotics that ranges are outside the limits.
- C** **Jenny Andrews.** I think it is fair to say that there is a lot of work being done on this issue. I think that Southmead are investing the actual disc contents of ciprofloxacin from various sources by eluting the drug and then undertaking HPLC.
- C** **from the audience.** We have also had a problem with vancomycin with a particular company and they have looked and said it was 4.6 and should have been at least 4.8.
- C.** **Professor Wise.** No one would have mentioned this with the Stokes' method, but with the standardised method people are being more critical and this is an excellent spin off.
- C** **Jenny Andrews.** We are always asking for zone data, even if it is only on a piece of paper or a table we do need this data for getting better acceptable limits. Bear in mind that the acceptable limits were generated from the first field study and there were only 20 laboratories, so if we can cast the net to over 100 laboratories submitting data we can actually reanalyse the data and look to see if those first zones of acceptability hold firm or need adjusting. We do need help as we only have 2 or 3 laboratories supplying information regularly.
- Q** We do the BSAC method and are constantly asked to look at antibiotics where there is no data eg topical sensitivities, azithromycin with *N. gonorrhoea* and in cases we are asked to look at rifampicin for streptococci and they are not there.
- C** **Professor Wise** As far as topical agents are concerned, no one has suggested breakpoints for these drugs. The concentration of the drug in the ointment, eye drop, eardrop etc is so high relative to the MIC, we do not know if resistance as defined by any standard method is meaningful or not. Even with urinary tract infections it is difficult, however there is some tentative evidence that current breakpoints are reliable. As far as topical agents are concerned we are all in the same boat, and the systemic breakpoint, if available, should be used for reporting. It may also be necessary to make a comment that '*for topical treatment the outcome is unknown*'.
- C** **Jenny Andrews.** In the case of azithromycin, Trevor Winstanley is currently looking at the recommendations for *N. gonorrhoea* and this includes testing azithromycin. Streptococci are on the wish list, at the moment we are looking at the effect of depth of media on the performance of control strains and the next study is the selection of a 'gold standard' medium. These are the priorities after which other needs will be dealt with. In the absence of recommendations, I suggest you do an MIC.

- Q** The only problem we have had is controlling zone sizes for mezlocillin.
- C** **Professor Wise.** I was not aware that mezlocillin was still available for use. I suggest you check the availability of the drug before spending a lot of time investigating the problem.
- Q** Your recommendation for anaerobes penicillin - is that a misprint? Do you know which manufacturers will supply this?
- A** **Jenny Andrews.** I do not have the Supplement with me, but Anna King wrote the chapter. It might be better if you discuss this with her directly.
- Q** I have a couple of questions. Firstly, is there any more news about zone sizes for alpha haemolytic streptococci? Are they still on the wish list? Is there any more news about testing *H. influenzae* against erythromycin and clarithromycin in CO₂?
- A** **Jenny Andrews.** The Working Party decided to incubate in CO₂ for two reasons. Firstly, we found that around 20-40% of pneumococci and haemophilus needed CO₂ to grow. CO₂ is not always needed for organisms that have been stored at -70°C, but it is needed for current clinical isolates. The first principles of the disc test are you must have an organism growing well after over night incubation. There is also evidence from Sue Hill and colleagues at Birmingham University that the pH in the lungs is round about pH 6. Putting these two things together we decided that testing should be undertaken in CO₂, however as you are aware this will affect the in-vitro activity of the macrolide antibiotics because of the acid conditions in the micro-environment of the test. Would you say macrolides are the best drugs against *H. influenzae*?
- C** **Professor Wise.** No. There are good clinical trials looking at clarithromycin where you get an unacceptably high failure rate to macrolides.
- Q** About the use of antibiotics in the veterinary field, one problem we had in practice was animals being brought in where the owner could not afford laboratory tests, so there was empirical treatment. What is the situation now?
- A** **Professor Wise.** It is only an expensive animal that would be investigated, for example a racehorse or cows.
- Q** Will there be any data for Coryneforms and Listeria species?
- A** **Jenny Andrews.** Michael Collins has a student who is looking at Coryneforms by BSAC methodology for his MSc project. So, yes we will have something in the future.
- C** **Professor Wise.** You said you have a lot of JKs, could you send them to Mr. Collins or to Jenny.
- Q** We have had a few problems with *E. coli* NCTC10418. We have been unable to get semi-confluent growth using a 1:100 dilution. Are most people using another the ATCC control?
- A** **Jenny Andrews.** We have just been doing a study and we have been using the NCTC and ATCC strains. We made up suspensions equivalent to a 0.5 MacFarland standard manually and then checked the density in a nephelometer, and we managed to get semi confluent growth. Are you finding the inoculum too heavy?
- R** No, too light.
- A** **Jenny Andrews.** I suggest you try a new control.
- Q** Are you going to suggest a *N. gonorrhoeae* control in the near future?
- A** **Jenny Andrews.** Again this is work that Trevor Winstanley is doing at the moment, which should be out by the end of the year. This is another MSc project.
- Q** With *Stenotrophomonas* you recommend 30°C but the *pseudomonas* control is incubated at 37°C. Is that correct?
- A** **Jenny Andrews.** Anna King in the BSAC Supplement recommended this.
- R** A couple were meropenem sensitive and they should all be resistant.
- R** **Jenny Andrews.** They are very difficult organisms; I think Anna suggests that imipenem is used for testing not meropenem. If you still have problems after switching to imipenem I suggest you contact Anna.

- C** **from the audience.** We had the same problem with meropenem and *Stenotrophomonas* picking up sensitivity where we should have had resistance, we just stopped testing. About the *E. coli* 10418, we do not use Vitek we make the suspension manually. We are also having problems in that we are getting a much lighter inoculum so we made a 1:10 dilution and we no longer have a problem. The problem is only with the NCTC *E. coli*.
- Professor Wise.** What is the reason for that?
- Jenny Andrews.** You have to remember that the 10418 and Oxford Staphylococcus are extremely susceptible to changes in growth conditions. Are the plates you use home made or purchased?
- R** Bought plates from Oxoid.
- R** **Jenny Andrews.** We have been using plates from Oxoid and have no problems, so it is hard to explain why it is not growing. I suggest getting a new freeze-dried culture from NCCLS.
- C** **from the audience.** We find with *E. coli* we need two beads to get the control to grow, instead of one for all the other organisms we use.
- Q** MICs and E tests. We find that in our laboratory we use E tests more and more. Firstly, because of the problem we have with resistance and secondly to confirm resistance of *N. gonorrhoeae* to penicillin and ciprofloxacin. For GCs we were sending them to the reference laboratory and having sensitivities confirmed by agar dilution where they use NCCLS interpretative criteria. We were having problems with our GU consultant because results from us by BSAC were different to those from the Reference Laboratory notably for penicillin and ciprofloxacin. Our laboratory was reporting moderately resistant and the Reference Laboratory reported moderately sensitive. They were not major differences but enough to annoy our GU consultants. To overcome this we thought we would use BSAC MIC breakpoints to interpret an E test MIC. However, I am concerned about how useful is an Etest for confirming an MIC, because for a NEQAS *S. pneumoniae* that had an MIC of 2 mg/L we reported an MIC of 0.75mg/L by E test. Obviously we got the category wrong. What should we do when we do not have the facility to do agar MICs?
- A** **Jenny Andrews.** We were recently debating this at the Working Party because it is confusing. Currently the insert with the kit recommends NCCLS methodology and interpretative criteria and these may be different to the BSAC breakpoints. MICs should generally be the same, however on occasions they are different for example Derek Brown says that MICs for *S. pneumoniae* to penicillin by Etest will be lower than by conventional agar methods. So this would explain the differences in result you have observed. I think you have to be aware that there are differences between all methods of testing and that there is never complete agreement. You have to decide whether those differences are significant to affect the outcome of the patient.
- C** **Professor Wise.** Derek Brown was of the opinion that results were usually very close to each other. However, an experienced person should do the reading of the results. What was the problem with the penicillin?
- R** Our disc results were not correlating exactly with the agar dilution results coming back from Bristol. Since we no longer send them to Bristol and now do E test, the zone diameters correlate with the Etest result. We also have a problem with control organism *Pseudomonas* 10662 and piperacillin/tazobactam. The zone diameters we get are below the limits of acceptability. I can send you this as it is recorded on the Aura zone reader.
- Q** Talking about E tests and doing disc testing for vancomycin and teicoplanin, you do not give recommendations for coagulase negative staphylococci. We currently use breakpoint technology and we have a cut off of 4 mg/L. Anything above 4 mg/L we test further by E test. One problem is that if you follow directions provided by AB Biodisk you get one answer that does not correlate with the reference laboratory results. We spoke Alan Johnson at the Reference Laboratory and he said that we should be using a larger inoculum and using brain heart infusion agar to test on. We asked AB Biodisk to look at this for us, but have not had a reply. I am concerned that if you are not using the Etest in the way the manufacturer recommends, then you are not meeting your requirements of SOPs.

- R Jenny Andrews.** We do not give recommendations for disc testing CNS to teicoplanin so I think there is some confusion. The method you describe using Brain Heart Infusion agar is for the detection of VISAs in *S. aureus* not for a routine MIC testing to vancomycin or teicoplanin. This is a dilemma because if you use Etest as the manufacturer recommends, you should use Mueller Hinton agar and the breakpoints are different.
- C from the audience.** The breakpoints are considerably different. If it was only one tube I would not be so worried, but sometimes there is 2 or 3 dilutions difference.
- R Jenny Andrews.** What you call resistant by BSAC breakpoints would be considered intermediate susceptibility by NCCLS. It creates a big problem.
- Professor Wise.** Do you believe that the country should firm up on our Visa/Gisa recommendations and testing?
- R Jenny Andrews.** Personally, I do not think that laboratories should spend a lot of time screening every *S. aureus*. In the past we spent a lot of time screening organisms large numbers of isolates from our hospital and other centres in the UK, and we found the screening methods very unreliable. Southmead hospital in Bristol where the first resistant organism was isolated, have more experience with VISAs than any other UK centre and I think they would agree that routine screening is a waste of resources and misleading. I spoke to Dr. Nick Brown a consultant microbiologist at Cambridge about their isolate and he said it was detected on the clinical picture because the patient failed therapy.
- Professor Wise.** Is it easy to detect a VISA?
- R Jenny Andrews.** You cannot detect VISAs by any disc testing method, so you have to do something different. Southmead have looked at gradient plates, and Hiramatsu's screening method and the results were very unreliable. They have found that the most reliable method is by population studies and this is not a test that would be undertaken by most diagnostic laboratories.
- Professor Wise.** I think Southmead's was a heterogeneous resistant strain and Cambridge's isolate was homogeneous.
- C from the audience.** We isolated a resistant organism at UCH. It was picked up by E test. It was a clinical failure, we sent it to Bristol and it came back heterogeneous resistance.
- Professor Wise.** Jenny do you think we are confident that we are picking up extended spectrum β -lactamases in diagnostic laboratories? Whatever shape and form whether they be the Tem series, ESBLs or CTXM series.
- R Jenny Andrews.** I think most laboratories use either cefpodoxime or ceftazidime as an indicator of resistance. However, cefpodoxime is better for detecting CTXM ESBLs.
- C from the audience.** There is an ESBL method on the Vitek, but the carbapenemases are missed.
- Professor Wise.** You only pick up half the carbapenemases at best this is another concern.
- Q** One of the problems with the recommendations is that for cefpodoxime a 5 **mg** disc is recommended but a 10 **mg** disc from Oxoid is supplied.
- A Jenny Andrews.** You have to remember that the 5 **mg** disc is used for therapeutic interpretation. For the test to detect ESBLs a 10 **mg** disc is used.
- Q** Can you recommend a manufacturer of cefpodoxime 5 **mg** discs?
- A Jenny Andrews.** I thought you could get them from Oxoid.
- Q** Are there any other kinds of resistances we should be screening for?
- Professor Wise.** Jenny said no to VISAs, though we may have to change our tune if things deteriorate; carbapenemases we have to get to grips with that sometime, particularly as I said there are going to be new carbapenems coming along, and this will act as a selecting pressure without doubt.
- Jenny Andrews.** We should look for quinolone resistance in things like GCs and Haemophilus. I think for Haemophilus we should be testing nalidixic acid as the kind of weaker member of the group. It might be the GC story again.
- Professor Wise.** Haemophilus when sensitive to ciprofloxacin, the MIC is 0.004 mg/L they are exquisitely sensitive. When they are resistant the MIC may only go up to 0.25 mg/L. You would like to know it was different; therefore a weaker drug like nalidixic acid should be used as recommended by the BSAC.

- Q** We are still finding problems with cefuroxime zone sizes for the sensitive Haemophilus control the zones are consistently small, by 1 or 2 mm. Is anyone else finding that?
- R** **Jenny Andrews.** Can you send me your data because we are looking at Haemophilus and we could look to see if we get the same results as yourselves?
- R** We wondered if it was the media and we get it ready poured from E & O. They have reassured that they are ISA based, I have not seen it confirmed in writing.
- Jenny Andrews.** We have done some work with Anna King and Derek Brown looking at different media for testing haemophilus and found that E & O (LabM base) was not as good as ISA. It might be the media base.
- R** I tried 10 plates from bioMerieux as well and that was marginally better. I was wondering whether anyone else was having problems?
- Jenny Andrews.** We have been looking at plates we have poured ourselves to a depth of 4mm and pre-poured plates from BioMerieux and Oxoid and they have compared favourably.
- Professor Wise.** As Jenny said, one of the things we are trying to do is get a 'gold standard' because there is a need for the performance of media to be standardized.
- Q** Are there any updates or information with testing levofloxacin with *S. pneumoniae*? Our consultants have emailed you about that. We are consistently getting zones less than 20.
- Jenny Andrews.** I forwarded your email to the people in Belfast who use the method all the time. Did you get a reply?
- R** No.
- Jenny Andrews.** I will contact them again for you.
- Professor Wise.** What problem do you have?
- R** The BSAC zone size is something like 20 mm; we are getting consistently less than that. The MICs are 0.5 to 0.75 mg/L and corresponding zones are 11 mm and up to 16 mm.
- C** **Professor Wise.** You mentioned MICs of 0.75 mg/L by Etest. One of the problems we had was quinolones and pneumococci and E test giving lower answers.
- R** **Jenny Andrews.** If you send us these organisms we can do MICs by conventional methods and we can compare the results.
- Q** To go back to discs. We have had problems with levofloxacin, vancomycin, ciprofloxacin and cefuroxime discs. Could this be related to supplier?
- R** **Jenny Andrews.** It may be, but we have not noticed problems using Oxoid discs and media.
- Q** We use nalidixic acid for GCs, what about other Neisseria?
- R** **Jenny Andrews.** Yes. If you were looking for low-level resistance that this will be, I would always go for nalidixic acid
- Q** I just wanted to ask how often people are testing their controls? How many people are doing Shewart plots and the whole control recommendations?
- R** **Jenny Andrews.** Most laboratories when they have introduced the method tend to test the controls every day. When they are more comfortable with testing they probably test weekly or if there is a new batch of media or plates. Some test once a fortnight. I think most people are using plots where you take 20 readings and take one in 20 being outside the acceptable limits as chance.

4.3 General discussion:

The merits of daily testing, weekly and fortnightly testing.

C Jenny Andrews. Some laboratories have experienced difficulties reporting the susceptibility of haemophilus to cefuroxime, ampicillin, amoxycillin, co-amoxyclav. We are currently re-viewing the zone diameter breakpoints and the amendments will be published on the web site in the next few weeks.

Professor Wise. I would like to conclude by talking about the future of susceptibility testing within Europe. The EUCAST group was originally chaired by Ian Phillips and was making little progress. Gunnar Kahlmeter from Sweden, who is also a member of the BSAC Working Party, now chairs the group and there is a very good relationship between the Swedish and British groups with an active exchange of data. He will have to accommodate 15 or so views from around Europe and my guess is that there is going to be some form of hybrid method recommended. Whether it will be more allied to the BSAC, Swedish or the NCCLS method, I do not know. I do not think it will be NCCLS because even the Chairperson of the NCCLS, Mary Jane Ferrari, states that if they were producing the NCCLS method now, they would not have the method they have at present, because there are problems with it. They would never have chosen Mueller Hinton medium or the inoculum, knowing what they know now. However, that is all water under the bridge.

It might be necessary to tweak the BSAC standardized method to bring it in line with 'The European method' whenever it is introduced, maybe 5 or 7 years ahead. However, I think the BSAC method will be accepted in some shape or form as a European method.